



STANDARD ARTICLE

Behavior in dogs with spontaneous hypothyroidism during treatment with levothyroxine

Alenka Hrovat¹  | Tiny De Keuster¹ | Hans S. Kooistra² | Luc Duchateau³ | Mark A. Oyama⁴  | Kathelijne Peremans⁵ | Sylvie Daminet¹

¹Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

²Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

³Biometrics Research Group, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

⁴Department of Clinical Studies-Philadelphia, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

⁵Department of Veterinary Medical Imaging and Small Animal Orthopedics, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

Correspondence

Alenka Hrovat, Pride Veterinary Centre, Riverside Road, Derby DE24 8HX, United Kingdom.

Email: alenka.hrovat@scarsdalevets.com

Background: Thyroid hormone supplementation anecdotally has been described as a valid treatment option for dogs with aggression-related problems. However, prospective, controlled, and blinded trials evaluating behavior and neurohormonal status in hypothyroid dogs during treatment with levothyroxine are lacking.

Objective: Levothyroxine supplementation will have a significant influence on the behavior and neurohormonal status of dogs with spontaneous hypothyroidism.

Animals: Twenty client-owned dogs diagnosed with spontaneous hypothyroidism.

Methods: This prospective study was to evaluate the behavior of dogs, which was screened at initial presentation, and after 6 weeks, and 6 months of treatment with levothyroxine (starting dosage 10 µg/kg PO q12h) using the standardized Canine Behavioral Assessment and Research Questionnaire (C-BARQ). At each time period, circulating serotonin and prolactin (PRL) concentrations were evaluated using a commercially validated ELISA kit and heterologous radioimmunoassay, respectively.

Results: After 6 weeks of thyroid hormone supplementation, C-BARQ scores demonstrated a significant increase in activity of hypothyroid dogs ($P < .01$). No significant change in any of the behavioral signs was observed after 6 months of treatment. No significant difference in circulating concentrations of serotonin ($P > .99$ and $P = .46$) and PRL ($P = .99$ and $P = .37$) were noted between the 6-week and 6-month periods compared with baseline.

Conclusions and Clinical Importance: The results of this study indicate increased activity of hypothyroid dogs after 6 weeks of thyroid hormone supplementation. None of the hypothyroid dogs in this cohort showed a significant change in any of the evaluated behavioral signs and neurohormonal status after 6 months of thyroid hormone supplementation.

KEYWORDS

canine, prolactin, serotonin, thyroid supplementation

1 | INTRODUCTION

Mental dullness and lethargy are 2 of the most frequently documented behavioral abnormalities in hypothyroid dogs, which typically

resolve within a few weeks of thyroid hormone supplementation.¹ A few authors also suggested an increase in irritability and unprovoked aggression towards animals and people in some hypothyroid dogs, which improved with combined levothyroxine and behavioral treatment.^{2–6} A recent randomized placebo-controlled study also described decreased owner-directed aggression after the start of thyroid hormone supplementation in “borderline hypothyroid” dogs.⁷ In contrast, recent case-controlled and cross-sectional studies failed to show abnormalities in several thyroid analytes in dogs with behavioral

Abbreviations: 5HT, 5-hydroxytryptamine; C-BARQ, Canine Behavioral Assessment and Research Questionnaire; CSF, cerebrospinal fluid; FT3, free triiodothyronine; FT4, free thyroxine; PRL, prolactin; TgAA, thyroglobulin antibodies; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; TT4, total thyroxine; TT3, total triiodothyronine.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2018 The Authors. *Journal of Veterinary Internal Medicine* published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.

and aggression-related problems.^{8,9} Based on these reports, a standardized evaluation of temperament and behavior and the effect of thyroid hormone supplementation on behavior in hypothyroid dogs is required.

Involvement of the serotonergic system in dogs with aggression has been established in several reports based on the measurement of serotonin concentrations in blood or cerebrospinal fluid (CSF), functional brain imaging, or by the successful use of drugs influencing serotonergic neurotransmission, namely selective serotonin reuptake inhibitors.^{10–16} Thyroid hormones appear to affect the concentrations of serotonin in blood and different brain regions and modulate serotonin turnover in the brain.^{17,18} These mechanisms have been studied extensively in humans and in animal models, but none of the studies has evaluated the effect of thyroid hormone supplementation on the serotonergic system in dogs.

Another neuroprotein affected by thyroid hormones is prolactin (PRL). In addition to its mammatropic and lactogenic properties, PRL also has a complex effect on neuroendocrine and behavioral adaptations, and has been associated with maternal aggression in female dogs.^{19,20} Thyrotropin-releasing hormone (TRH) has been shown to stimulate PRL gene expression and PRL release from lactotrophs in a dose-dependent manner.²¹ Hyperprolactinemia and thyroid-stimulating hormone (TSH) at the high end of the reference range also have been documented in women diagnosed with hypothyroidism; however, further studies failed to document aggressive behavior or mood disorders in these groups of women.^{21,22} In dogs, aggression associated with hyperprolactinemia has mostly been associated with pseudopregnancy, although no study has examined PRL concentrations in otherwise aggressive dogs.²⁰

Based on this information, the first aim of our prospective study was to evaluate the behavior of dogs with spontaneous hypothyroidism during treatment with levothyroxine using a standardized canine behavioral questionnaire. A second aim was to evaluate circulating serotonin and PRL concentrations during thyroid hormone supplementation.

2 | MATERIALS AND METHODS

2.1 | Animals

Client-owned dogs of various breeds, ages, and body weights were prospectively included in this study. Dogs consisted of both first opinion and referral cases (ie, dogs presented or referred to the Small Animal Department, Faculty of Veterinary Medicine, Ghent University, and Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University). For dogs recruited from first opinion practices and for referral cases, the same exclusion and inclusion criteria applied. The owners of the dogs were blinded to the aims of the study and were told that the objective of the study was to investigate the quality of life of dogs with hypothyroidism during thyroid hormone supplementation.

To be included in the study, dogs had to have clinical and laboratory variables consistent with hypothyroidism (ie, physical examination

[PE] findings and routine laboratory examinations including hematology and serum biochemistry).

All participating practitioners received an information brochure and checklist summarizing the aims of the study and the inclusion and exclusion criteria. Once the checklist was reviewed and the dogs fulfilled all of the inclusion criteria, participating practices were provided with the sampling material and instructions on the sampling, processing, and storage of samples. Compliance with the study criteria was controlled by means of regular follow-up phone calls and email reminders.

Disorders unrelated to hypothyroidism were ruled out based on thorough history, PE, hematology, and serum biochemistry profiles. Dogs were excluded from the study if they had received any medication within 2 months before inclusion and throughout the duration of the study except the following: flea and heartworm prophylaxis, routine vaccination, topical antifungals (eg, ketoconazole, itraconazole), PO antibiotics except sulfonamides, and topical ear solutions that did not contain glucocorticoids. Pregnant dogs and dogs suffering from any concurrent systemic disorder also were excluded.

2.2 | Diagnosis and treatment of hypothyroidism

The diagnosis of hypothyroidism had to be confirmed by finding a circulating total thyroxine (TT4) concentration $<1.2 \mu\text{g/dL}$ and a circulating TSH concentration $>0.6 \text{ ng/mL}$. In cases in which the TT4 and TSH concentrations were not conclusive, the diagnosis was confirmed by a TSH stimulation test or scintigraphic assessment of the thyroid function. The TSH response test was performed using $150 \mu\text{g}$ of freshly reconstituted or frozen recombinant human TSH as previously described.^{23–25} For scintigraphic assessment of thyroid function for diagnosing hypothyroidism, dogs with very low or absent technetium-99m pertechnetate uptake during thyroid scintigraphy were considered hypothyroid.²⁶

After the diagnosis was confirmed (T_0), all dogs were started on treatment with levothyroxine sodium (Forthyron; Eurovet Animal Health B.V., Bladel, The Netherlands; starting dosage, $10 \mu\text{g/kg}$ PO q12h). Follow-up appointments were scheduled at 6 weeks (T_1) and 6 months (T_2) after the start of treatment. The goal of treatment was to obtain a peak TT4 concentration, 2–4 hours after administration of levothyroxine, at the high end of or slightly above the upper limit of the reference interval. For therapeutic monitoring, if levothyroxine was given at the time of feeding, the same protocol was followed on the day of testing. If dose adjustment at 6 weeks was needed, it was left at the discretion of the clinician, and another control was planned a month later.

2.3 | Evaluation of behavior

The behavior of dogs was evaluated at T_0 , T_1 , and T_2 by having the owners complete a hard copy of the Canine Behavioral Assessment and Research Questionnaire (C-BARQ). The C-BARQ is a standardized owner-completed online questionnaire (<http://www.cbarq.org>) designed to provide quantitative assessments of numerous behavioral and temperamental characteristics of dogs.^{27–30} The questionnaire is comprised of 7 general behavioral categories (ie, sociability, trainability,

aggression, fear and anxiety, excitability, separation-related behavior, attachment and attention-seeking behavior, and miscellaneous) and 101 scored questions evaluating dogs' typical responses to a variety of everyday situations, stimuli, and events in their environment.²⁷ Owners assess either the frequency or the severity of the behavioral characteristics using a 5-point ordinal scale, with higher scores usually being less favorable.²⁷⁻³⁰ For correct use of the scale, each section of the questionnaire includes a brief explanation of typical behavioral signs associated with each behavioral category that owners can recognize and use when scoring their dogs.²⁷ Documented behavioral characteristics are then further compared to the behavioral characteristics of all dogs and the dog's own breed.^{27,29,30}

Hard copies of CBARQ were available in 4 languages (Dutch, German, French, and English) allowing the owners to fill in the questionnaire in their native language.

2.4 | Blood sampling and storage

Blood was collected at T₀, T₁, and T₂. Because of daily variations of serotonin, blood samples were collected between 9 and 11 AM. Ten milliliters of blood was collected from the jugular vein and divided between serum and heparin tubes. During the initial presentation, 1 aliquot of ethylenediaminetetraacetic acid blood also was collected for hematology. Serum and heparin tubes were centrifuged and further aliquoted. One aliquot of serum was used for rapid monitoring of TT4 concentration, performed by each of the collaborating institutions and veterinary practices individually. Additional aliquots of serum and heparin were frozen immediately at -20°C. For storage >2 weeks, samples were stored at -80°C. Frozen serum aliquots were used for measurement of TT4, TSH, and serotonin. Serum TT4 and TSH concentrations were determined using a previously validated commercially available solid-phase, chemiluminescent competitive immunoassay (Immulite 2000 Canine TSH and Total T4; Gwynedd, United Kingdom).^{31,32} Frozen heparinized samples used for the measurement of PRL were transported for analysis to Utrecht University on dry ice. Upon arrival, the samples were stored at -80°C until being analyzed.

2.5 | Serotonin and PRL measurement

Serum serotonin or 5-hydroxytryptamine (5HT) concentration was measured with the Serotonin ELISA Kit (DDD) using a commercial enzyme immunoassay technique following the manufacturer's instructions. Measurement of serotonin was performed at the Department of Anatomy, Histology with Embryology and Cytology, Institute of Preclinical Sciences, Veterinary Faculty, University of Ljubljana. All samples were run in duplicate. The assay was validated for use in canine samples, and serotonin was calculated and expressed as previously described.³³ Briefly, canine serum was spiked with increasing concentrations of 5HT. Linearity and parallelism were assessed using spiked canine samples and manufacturer controls for 5 different concentrations. Recovery was assessed as the percentage of recovered 5HT-spiked canine samples vs expected 5HT at 6 different concentrations.³³

Plasma PRL concentration was measured at Utrecht University by using a previously validated heterologous radioimmunoactive assay.³⁴ The intra-assay and inter-assay coefficients of variation were 3.5% and 11.5%, respectively, and the lower limit of detection was 0.8 µg/L.

2.6 | Statistical analysis

The data were analyzed using commercial statistical software (GraphPad Prism v7.0; GraphPad Software, La Jolla, California). Because the data were not normally distributed, the Wilcoxon signed-rank test for paired samples was applied to compare serum serotonin and PRL concentrations among T₀, T₁, and T₂. For each dog, behavior before and on 2 occasions after the start of treatment was compared by calculating an average score on all questions within each behavioral category at each of the 3 occasions; a paired *t* test was used for comparison. Bonferroni's correction was applied to the data to account for multiple pairwise comparisons resulting in a comparison-wise significance level of 0.05/3 = 0.016.

3 | RESULTS

3.1 | Study population

Twenty dogs met the inclusion criteria. Fourteen of 20 dogs were included from first opinion practices, and the remaining 6 dogs were seen at the referral hospitals of University of Ghent and Utrecht. Fifteen dogs had circulating TT4 and TSH concentrations consistent with primary hypothyroidism. In 2 patients, a TSH stimulation test, and, in 3 patients, thyroid scintigraphy, were used to confirm hypothyroidism. The median age of dogs was 5.8 years (range, 2.5-11.3). There were 7 sexually intact females, 6 spayed females, 3 sexually intact males, and 4 neutered males. Breeds included 3 crossbreeds, 2 American Cocker Spaniels and Labrador Retrievers, and 1 each of Doberman, Border Collie, Bullmastiff, Bavarian Mountain Hound, Golden Retriever, Brittany Spaniel, English Cocker Spaniel, Bull Terrier, Wire-haired Pointing Griffon, Belgian Shepherd, Airedale Terrier, Shetland Sheepdog, and Basset Fauve De Bretagne. No abnormalities were observed in routine hematological variables.

Of the biochemical variables, only the serum concentrations of cholesterol (median, 528.9 mg/dL; range, 189.1-965.3 mg/dL; reference range: 111.9-386.1 mg/dL; N = 15) and triglycerides (median, 283.1 mg/dL; range, 35.4-1398.2 mg/dL; reference interval: 0-150.4 mg/dL; N = 14) were increased. For all other variables, results were within their respective reference intervals.

The median TT4 concentration was below the reference interval at T₀ and within the reference interval (0.5-3.4 µg/dL) at T₁ and T₂. The median circulating TT4 concentrations at T₁ (3 µg/dL; range, 0.5-5.9 µg/dL; N = 17) and T₂ (2.7 µg/dL; range, 1.6-6.4 µg/dL; N = 16) were significantly higher (*P* ≤ .001) compared with that at T₀ (< 0.5 µg/dL; range, < 0.5-2 µg/dL; N = 17). The median TT4 concentrations at T₁ and T₂ did not differ significantly (*P* = .44). Five dogs failed to achieve euthyroidism at T₁ and 2 dogs at T₂.

The median TSH concentration at T_0 (1.5 ng/mL; range 0.1–12 ng/mL; $N = 17$) was significantly higher than those at T_1 (0.12 ng/mL; range, 0.03–3.2 ng/mL; $N = 17$; $P < .008$) and T_2 (0.1 ng/mL; range, 0.01–0.9 ng/mL; $N = 16$; $P < .0001$). The TSH concentrations between T_1 and T_2 did not differ significantly ($P = .13$).

3.2 | Evaluation of behavior

Results of the C-BARQ analysis at T_0 , T_1 , and T_2 are shown in Table 1. At T_1 compared to T_0 , an overall significant increase ($P < .01$) in 1 of 7 behavioral categories (ie, activity levels) was documented. At T_2 , no significant changes in any of the behavioral categories were observed when compared with T_0 and T_1 .

3.3 | Measurement of serotonin and PRL

The results of the serotonin and PRL concentrations are shown in Figure 1. The median serum serotonin concentration at T_0 (2221 ng/mL; range, 426–2979 ng/mL) did not differ significantly from those at T_1 (1810 ng/mL; range, 284–2810 ng/mL; $P > .99$) and at T_2 (1739 ng/mL; range, 226–2836; $P = .46$). Also, no significant difference ($P = .46$) was noted in the serotonin concentrations between T_1 and T_2 . Similarly, the median plasma PRL concentration at T_0 (3.3 ng/mL; range, 1.4–6.4 ng/mL) did not differ significantly from that at T_1 (3.1 ng/mL; range, 1.9–7.4 ng/mL; $P = .99$) and T_2 (3.1 ng/mL; range, 2.0–12.9 ng/mL; $P = .37$). Also, no significant difference ($P = .30$) was noted in PRL concentrations between T_1 and T_2 .

4 | DISCUSSION

Our results documented a significant increase in activity in hypothyroid dogs after 6 weeks of thyroid hormone supplementation but failed to show any changes in evaluated behavioral signs during 6 months of treatment. Furthermore, serum serotonin and plasma PRL concentrations remained unchanged during thyroid hormone supplementation.

Lethargy, mental dullness, and inactivity are the most commonly observed clinical signs in hypothyroid dogs and are associated with decreased metabolic rate.¹ Thyroid hormone deficiency leads to a decrease in body energy consumption followed by decreased activity levels and lethargy.¹ Appropriate thyroid hormone supplementation

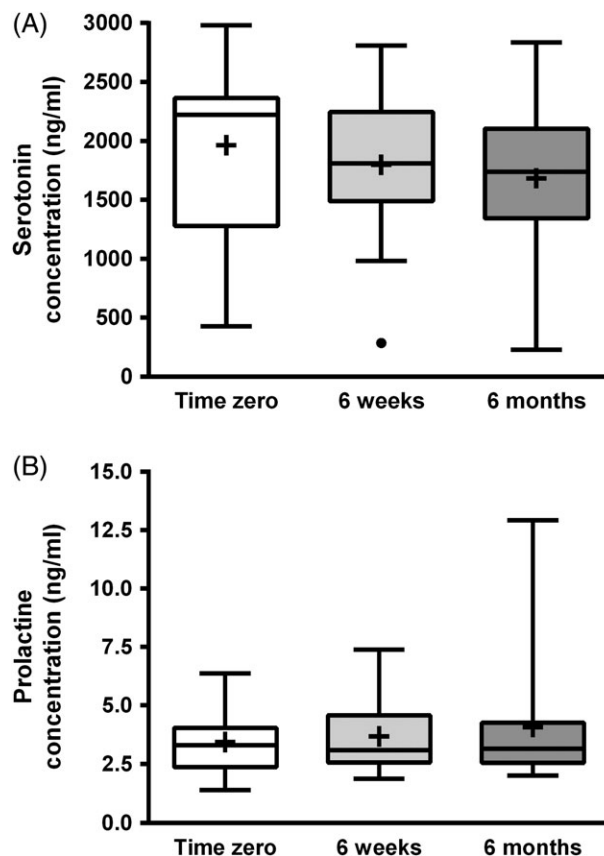


FIGURE 1 Box and whisker plots of (A) serum serotonin concentration of dogs diagnosed with spontaneous hypothyroidism before (T_0 ; $N = 14$) and after thyroid supplementation with levothyroxine at 6 weeks (T_1 ; $N = 16$) and 6 months (T_2 ; $N = 14$) after the start of treatment and (B) box and whisker plots of plasma prolactin concentration of dogs with primary hypothyroidism before (T_0 ; $N = 14$) and after thyroid hormone supplementation with levothyroxine at 6 weeks (T_1 ; $N = 18$) and 6 months (T_2 ; $N = 15$) after the start of treatment. Each box and whisker plot illustrates the median (line in the middle), mean (+), 25th and 75th percentiles (top and bottom of each box); whiskers extend from the 5th and 95th percentile values

therefore is expected to improve activity levels and mental alertness of patients within a few weeks of treatment.³⁵ A marked increase in activity levels is usually the earliest improvement in clinical signs.³⁵ In humans, hypothyroidism commonly is accompanied by decreased

TABLE 1 Analysis of the C-BARQ of dogs diagnosed with primary hypothyroidism before the treatment with levothyroxine (T_0 ; $N = 20$), 6 weeks (T_1 ; $N = 19$) and 6 months (T_2 ; $N = 17$) after initiation of treatment^a

Behavioral category	T_0 and T_1			T_0 and T_2			T_1 and T_2		
	Mean	95% CI	P	Mean	95% CI	P	Mean	95% CI	P
Training	−0.15	−0.27 to 0.06	.20	−0.09	−0.27 to 0.10	.33	0.04	−0.14 to 0.21	.66
Aggression	−0.23	−0.46 to −0.01	.04	−0.18	−0.40 to 0.04	.10	0.05	−0.14 to 0.24	.57
Fear	−0.22	−0.59 to 0.16	.24	0.02	−0.17 to 0.21	.81	0.21	−0.15 to 0.58	.23
Separation-related anxiety	0.01	−0.19 to 0.20	.95	0.01	−0.23 to 0.25	.95	0.02	−0.18 to 0.24	.80
Excitement	−0.35	−0.69 to −0.2	.04	−0.20	−0.57 to 0.18	.28	0.14	−0.18 to 0.47	.37
Attachment and attention seeking	−0.04	−0.40 to 0.32	.82	0.11	−0.29 to 0.51	.57	0.14	−0.16 to 0.43	.34
Activity level	−0.79	−1.38 to −0.20	.01 ^b	−0.44	−1.35 to 0.47	.32	0.37	−0.42 to 1.17	.33

^a Differences in behavior between T_0 , T_1 , and T_2 are expressed as mean and 95% confidence interval (CI).

^b Significant difference ($P < 0.01$).

cognition, drowsiness, slower thought processes, and lethargy.^{18,36,37} Several, but not all, behavioral disorders in humans diagnosed with hypothyroidism improve with thyroid hormone supplementation.^{38–42} Genetic factors and thyroid autoimmunity might determine individual response to thyroid hormone supplementation in humans and dogs.^{38,43}

For evaluation of behavior in our study, we used a standardized questionnaire as opposed to behavioral consultation by a specialist. A behavioral consultation would provide more details about the dogs' behavior and would enable us to follow up specific behavioral signs from distant and recent history. However, statistical analysis of such detailed information in a large number of dogs would not be possible. The CBARQ used in our study currently is the only standardized owner-based behavioral questionnaire allowing examination and overview of a large number of behavioral signs, creating data amenable to statistical analysis.^{27–29,44,45}

With CBARQ, our results contradict anecdotal reports supporting the use of thyroid hormones for the treatment of aggression in dogs.^{2–6} The majority of these reports either failed to conclusively document hypothyroidism or failed to provide follow-up information on the type and frequency of aggressive signs in dogs that achieved euthyroidism with thyroid hormone supplementation.^{2–6} Furthermore, some dogs experienced improvement of aggressive behavior but failed to achieve euthyroidism.² Moreover, all these dogs were treated using behavioral treatment together with thyroid hormone supplementation, which challenges the assumption that the improvement in behavioral abnormalities could be solely attributable to thyroid hormone supplementation.^{2–6} A recent prospective randomized placebo-controlled study documented a decreased incidence of owner-directed aggression of dogs with “suboptimal” thyroid function receiving thyroid hormone supplementation.⁷ This group of dogs showed unprovoked owner-directed aggression several times a week and had at least 1 nonspecific clinical sign that also may be seen in dogs with hypothyroidism. In addition to clinical abnormalities, their thyroid hormone concentrations (free T₄ [fT₄], TT₄, total T₃ [TT₃], and free T₃ [fT₃]) were considered to be low or at the low end of the reference interval.⁷ Although this study was the first prospective study that evaluated the use of thyroid hormones for the treatment of aggression in dogs without concurrent behavioral treatment, the assays used for measurement of serum concentrations of fT₄ and TSH were not specified. Furthermore, TT₄ concentration might decrease below the reference interval as much as 20% of the time in euthyroid dogs and could be affected by time of the day, breed, age, season, temperature, nonthyroidal illnesses, and drugs.^{46–50}

Thyroid status also affects the turnover of monoamines, specifically serotonin, in several centers of the central nervous system.^{17,18} Serotonin has been associated with what has previously been called “dominance aggression” in dogs, and the serotonin 2A receptor has been demonstrated to be a valid biomarker for aggressive and anxious behavior in dogs.^{10–13,15,51,52} In our study, the serum concentration of serotonin during thyroid supplementation was not significantly different from baseline. Studies in rats have shown a lesser effect of thyroid status on serotonin turnover in the adult as opposed to the neonatal brain.¹⁸ In humans, the role of thyroid hormones on serotonin

concentrations and responsiveness to serotonin-modulating drugs has not been consistently demonstrated.³⁸

Serotonin in the blood predominantly is stored in platelets and is easily released during sample preparation.⁵³ In this study, serotonin was measured in serum using an ELISA test that has been validated previously for dogs.³³ Although the concentration of neurotransmitters is best measured in the CSF, in humans, using novel methods, good correlation between platelet and CSF concentrations was shown.⁵⁴ Although these techniques have not been validated for dogs, studies comparing the serotonin concentrations of aggressive and nonaggressive dogs effectively managed to document significant differences in the serotonin concentrations using serum.^{11,13} With ELISA, serotonin in dogs showing aggressive behavior also has been reliably evaluated in plasma, platelets, and CSF.^{55,56}

Daily fluctuations in the circulating serotonin concentration are well recognized.⁵⁷ Although advanced laboratory techniques manage to minimize the effect of these fluctuations on the overall measured serotonin concentration, these techniques are not currently validated in dogs.⁵⁴ To avoid the effect of daily fluctuations on serotonin measurements, all blood collections were scheduled at the same daily time points, between 9 and 11 AM. Daily variations of serotonin in dogs have not been examined, but have been described in young horses, in which the highest daily concentrations of serotonin were measured in platelet-poor plasma in the late afternoon and the lowest concentrations occurred during the early morning hours.⁵⁸ Similar to horses, humans with depression had their lowest serotonin concentrations measured during morning hours and the highest concentrations measured in the afternoon.⁵⁹

Another neuroprotein that has been shown to be affected by thyroid hormones is PRL.¹⁹ In our study, PRL concentrations after 6 weeks and 6 months of thyroid supplementation were not significantly different compared to concentrations before treatment. Prolactin was measured in plasma using a heterologous radioimmunoassay, which has been validated previously in dogs.³⁴ It has long been recognized, based on classic experiments, that acute injection of serotonin or its precursor, 5-hydroxytryptophan, stimulates PRL release.⁶⁰ Individuals who have a low PRL response to serotonergic agonists express more frequent aggressive signaling compared with individuals with a high PRL response.⁶¹ In addition to serotonin, TRH also has been shown to stimulate PRL secretion and PRL gene expression.¹⁹ With thyroid hormone supplementation, TSH and TRH are normally down-regulated via feedback mechanisms mediated by thyroid hormones.⁶² With regard to TRH-associated release of PRL, decreased TRH would be expected to result in a decreased PRL concentration and consequently decreased aggression. Thyrotropin-releasing hormone was not measured in our study, and PRL remained unchanged after thyroid hormone supplementation. Although a direct link among thyroid hormones, serotonin, TRH, and PRL was not examined in our study, based on our findings and in contrast to older reports, PRL might not play such an important role in the modulation of dogs' behavior as initially assumed.¹⁹ Similar contradictory conclusions are found in the human medical literature, with some studies documenting hyperprolactinemia in hypothyroid women with aggressive behavior or mood disorders and other studies failing to yield comparable conclusions.^{21,22}

Our study contributes important insight into the role of thyroid hormone supplementation on behavior in dogs. However, the main limitations are a small number of dogs, lack of a control group, and use of a standardized behavioral questionnaire as opposed to individual consultation with a behaviorist. The CBARQ provides a quantitative score for a specific predetermined set of behavioral signs, and therefore, in a setting such as this one in which a change or a follow-up of behavior is required, small or specific behavioral alterations could go undetected.²⁷ Despite being used in different research settings, to our knowledge, the sensitivity and specificity of this tool for the examination of different behavioral characteristics of dogs have not been examined. The lack of behavioral changes after 6 months of thyroid supplementation in this group of dogs may be ascribed to the lack of sensitivity of the behavioral questionnaire. The CBARQ also is an owner-based questionnaire; therefore, a lack of objective and consistent evaluation of the behavior during follow-up of these dogs is possible. The owners completed a new questionnaire during each visit without seeing their scores from the previous questionnaire. It would be interesting to speculate if the scoring at 6 months would be different if the owners were allowed to directly compare the 6-month and the 6-week questionnaires. Some of the 6-month questionnaires contained owners' comments indicating that no change in the overall behavior was documented since the last visit, but the scoring of the behavioral categories to which the owners referred was different. Furthermore, when evaluating the change in the behavior at 6 months, owners were most likely comparing dogs' behavior with the 6-week period and not the time before treatment was started. This could explain why the activity level, which initially was increased, was considered as being not substantially increased at 6 months' time.

None of the dogs in our study had abnormal behavior or abnormal concentrations of serotonin or PRL that would allow us to observe potential improvement of these abnormalities with thyroid hormone supplementation. In this regard, we cannot exclude a beneficial effect of levothyroxine for treatment of dogs with increased irritability and unprovoked aggression towards animals and people. Future studies should aim at including hypothyroid dogs with these problems.

Observational studies in veterinary medicine and studies in human medicine, although inconsistent, have documented an influence of thyroid antibodies in mental disorders and individual response to thyroid hormone supplementation.^{38,43} Measurement of thyroglobulin antibodies (TgAA) and free T4 in this cohort of dogs and future studies would be interesting and also useful in dog breeds known to be affected by autoimmune thyroiditis (ie, Doberman Pinchers), 1 of which also was included in our study. However, to properly establish the effect of autoimmune thyroid disease on brain function, behavior, and thyroid hormone supplementation, advanced neuroimaging and measurement of antibody reactivity against brain tissue would be required and can be explored in future research.^{63,64} It also would be useful to perform a neurological examination in this cohort of dogs because it would allow us to evaluate the status of the central and peripheral nervous system, both of which can be affected by hypothyroidism. However, because the majority of dogs included in our study were recruited from first opinion practices, doing so would not be possible and would not provide an additional therapeutic benefit for these dogs.

Although larger studies and use of a control group are needed, our study provided a standardized evaluation of the behavior and neurohormonal status of dogs treated with thyroid hormones.

ACKNOWLEDGMENTS

The authors thank Dr James Serpell, author and founder of the CBARQ questionnaire, for the permission to use the CBARQ in this study and providing hard copies of the questionnaire in Dutch, German, French, and English, collaborating general practitioners for help with recruitment; Elke Deutekom, a final year student of the Faculty of Veterinary Medicine, Utrecht University, for help with the recruitment of dogs; Prof Milka Vrecl and Prof Gregor Fazarinc from the Department of Anatomy, Histology with Embryology and Cytology, Institute of Preclinical Sciences, Veterinary Faculty, University of Ljubljana for assistance and permission to perform the measurement of the serotonin at the aforementioned institute. This work was performed at the Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium, and Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands. Part of this work was presented as an abstract at the annual ECVIM-CA congress in Mainz, Germany, September 10–12, 2015.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

INSTITUTIONAL ANIMAL CARE AND USE OF COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Granted by the Ethical Committee, Faculty of Veterinary Medicine, Ghent University.

ORCID

Alenka Hrovat  <https://orcid.org/0000-0003-4319-8902>

Mark A. Oyama  <https://orcid.org/0000-0002-5218-9226>

REFERENCES

1. Scott-Moncrieff JC. Clinical signs and concurrent diseases of hypothyroidism in dogs and cats. *Vet Clin North Am Small Anim Pract.* 2007;37:709-722.
2. Beaver BV, Haug LI. Canine behaviors associated with hypothyroidism. *J Am Anim Hosp Assoc.* 2003;39:431-434.
3. Beaver BV. Animal behavior case of the month. *J Am Vet Med Assoc.* 1993;203:974-975.

4. Dodman NH, Mertens PA, Aronson LP. Animal behavior case of the month. Dogs were evaluated because of aggression. *J Am Vet Med Assoc.* 1995;207:1168-1171.
5. Fatjó J, Amat M, Manteca X. Animal behavior case of the month. Aggression in dogs. *J Am Vet Med Assoc.* 2003;223:623-626.
6. Fatjó J, Stub C, Manteca X. Four cases of aggression and hypothyroidism in dogs. *Vet Rec.* 2002;151:547-548.
7. Dodman NH, Aronson L, Cottam N, Dodds JW. The effect of thyroid replacement in dogs with suboptimal thyroid function on owner-directed aggression: a randomized, double-blind, placebo-controlled clinical trial. *J Vet Behav Clin Appl Res.* 2013;8:225-230.
8. Carter GR, Scott-Moncrieff JC, Luescher AU, Moore G. Serum total thyroxine and thyroid stimulating hormone concentrations in dogs with behavior problems. *J Vet Behav Clin Appl Res.* 2009;4:230-236.
9. Radosta LA, Shofer FS, Reisner IR. Comparison of thyroid analytes in dogs aggressive to familiar people and in non-aggressive dogs. *Vet J.* 2012;192:472-475.
10. Reisner IR, Mann JJ, Stanley M, Huang YY, Houpt KA. Comparison of cerebrospinal fluid monoamine metabolite levels in dominant-aggressive and non-aggressive dogs. *Brain Res.* 1996;714:57-64.
11. Cakiroglu D, Meral Y, Sancak AA, Cifti G. Relationship between the serum concentrations of serotonin and lipids and aggression in dogs. *Vet Rec.* 2007;161:59-61.
12. Rosado B, García-Belenguer S, Palacio J, Chacón G, Villegas A, Alcalde AI. Serotonin transporter activity in platelets and canine aggression. *Vet J.* 2010;186:104-105.
13. Rosado B, García-Belenguer S, León M, et al. Effect of fluoxetine on blood concentrations of serotonin, cortisol and dehydroepiandrosterone in canine aggression. *J Vet Pharmacol Ther.* 2011;34:430-436.
14. Vermeire ST, Audenaert KR, Dobbeleir AA, de Meester RH, de Vos FJ, Peremans KY. Evaluation of the brain 5-HT_{2A} receptor binding index in dogs with anxiety disorders, measured with 123I-5I-R91150 and SPECT. *J Nucl Med.* 2009;50:284-289.
15. Vermeire S, Audenaert K, De Meester R, et al. Neuro-imaging the serotonin 2A receptor as a valid biomarker for canine behavioural disorders. *Res Vet Sci.* 2011;91:465-472.
16. Vermeire S, Audenaert K, De Meester R, et al. Serotonin 2A receptor, serotonin transporter and dopamine transporter alterations in dogs with compulsive behaviour as a promising model for human obsessive-compulsive disorder. *Psychiatry Res.* 2012;201:78-87.
17. Bauer M, London ED, Silverman DH, et al. Thyroid, brain and mood modulation in affective disorder: insights from molecular research and functional brain imaging. *Pharmacopsychiatry.* 2003;36:215-221.
18. Bauer M, Heinz A, Whybrow PC. Thyroid hormones, serotonin and mood: of synergy and significance in the adult brain. *Mol Psychiatry.* 2002;7:140-156.
19. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. *Physiol Rev.* 2000;80:1523-1631.
20. Jöchle W. Abnormal behavior and adaptation problems in dogs and cats and their pharmacologic control. *Tierarztl Prax Ausg K Kleintiere Heimtiere.* 1998;26:410-421.
21. Meier C, Christ-Crain M, Guglielmetti M, Huber P, Staub JJ, Müller B. Prolactin dysregulation in women with subclinical hypothyroidism: effect of levothyroxine replacement therapy. *J Am Thyroid Assoc.* 2003;13:979-985.
22. Barry JA, Moran E, Parekh HS, Morewood T, Thomas M, Hardiman PJ. Prolactin and aggression in women with fertility problems. *J Obstet Gynaecol.* 2014;34:605-610.
23. De Roover K, Duchateau L, Carmichael N, et al. Effect of storage of reconstituted recombinant human thyroid-stimulating hormone (rhTSH) on thyroid-stimulating hormone (TSH) response testing in euthyroid dogs. *J Vet Intern Med.* 2006;20:812-817.
24. Daminet S, Fifle L, Paradis M, Duchateau L, Moreau M. Use of recombinant human thyroid-stimulating hormone for thyrotropin stimulation test in healthy, hypothyroid and euthyroid sick dogs. *Can Vet J.* 2007;48:1273-1279.
25. Boretti FS, Sieber-Ruckstuhl NS, Wenger-Riggenbach B, et al. Comparison of 2 doses of recombinant human thyrotropin for thyroid function testing in healthy and suspected hypothyroid dogs. *J Vet Intern Med.* 2009;23:856-861.
26. Diaz Espineira MM, Mol JA, Peeters ME, et al. Assessment of thyroid function in dogs with low plasma thyroxine concentration. *J Vet Intern Med.* 2007;21:25-32.
27. Hsu Y, Serpell JA. Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. *J Am Vet Med Assoc.* 2003;223:1293-1300.
28. Segurson SA, Serpell JA, Hart BL. Evaluation of a behavioral assessment questionnaire for use in the characterization of behavioral problems of dogs relinquished to animal shelters. *J Am Vet Med Assoc.* 2005;227:1755-1761.
29. Duffy DL, Hsu Y, Serpell JA. Breed differences in canine aggression. *Appl Anim Behav Sci.* 2008;114:441-460.
30. Serpell JA, Hsu Y. Effects of breed, sex, and neuter status on trainability in dogs. *Anthrozoos.* 2005;18:196-207.
31. Marca MC, Loste A, Orden I, González JM, Marsellá JA. Evaluation of canine serum Thyrotropin (TSH) concentration: comparison of three analytical procedures. *J Vet Diagn Invest.* 2001;13:106-110.
32. Panakova L, Koch H, Kolb S, Mueller RS. Thyroid testing in Sloughis. *J Vet Intern Med.* 2008;22:1144-1148.
33. Arndt JW, Reynolds CA, Singletary GE, Connolly JM, Levy RJ, Oyama MA. Serum serotonin concentrations in dogs with degenerative mitral valve disease. *J Vet Intern Med.* 2009;23:1208-1213.
34. Okkens AC, Dieleman SJ, Bevers MM, Willemsse AH. Evidence for the non-involvement of the uterus in the lifespan of the corpus luteum in the cyclic dog. *Vet Q.* 1985;7:169-173.
35. Dixon RM, Reid SWJ, Mooney CT. Treatment and therapeutic monitoring of canine hypothyroidism. *J Small Anim Pract.* 2002;43:334-340.
36. Bauer M, Goetz T, Glenn T, Whybrow PC. The thyroid-brain interaction in thyroid disorders and mood disorders. *J Neuroendocrinol.* 2008;20:1101-1114.
37. Hage MP, Azar ST. The link between thyroid function and depression. *J Thyroid Res.* 2012;2012:590648.
38. Bunevicius R, Prange AJ. Thyroid disease and mental disorders: cause and effect or only comorbidity? *Curr Opin Psychiatry.* 2010;23:363-368.
39. Joffe RT. Hormone treatment of depression. *Dialogues Clin Neurosci.* 2011;13:127-138.
40. Bauer M, Berman S, Stamm T, et al. Levothyroxine effects on depressive symptoms and limbic glucose metabolism in bipolar disorder: a randomized, placebo-controlled positron emission tomography study. *Mol Psychiatry.* 2016;21:229-236.
41. Labad J, Barbero JD, Gutiérrez-Zotes A, et al. Free thyroxine levels are associated with cognitive changes in individuals with a first episode of psychosis: a prospective 1-year follow-up study. *Schizophr Res.* 2016;171:182-186.
42. Barbero JD, Gutiérrez-Zotes A, Montalvo I, et al. Free thyroxine levels are associated with cognitive abilities in subjects with early psychosis. *Schizophr Res.* 2015;166:37-42.
43. Dodds WJ. Estimating disease prevalence with health surveys and genetic screening. *Adv Vet Sci Comp Med.* 1995;39:29-96.
44. van den Berg SM, Heuven HCM, van den Berg L, Duffy DL, Serpell JA. Evaluation of the C-BARQ as a measure of stranger-directed aggression in three common dog breeds. *Appl Anim Behav Sci.* 2010;124:136-141.
45. Duffy DL, Serpell JA. Effects of early rearing environment on behavioral development of guide dogs. *J Vet Behav Clin Appl Res.* 2009;4:240-241.
46. Miller AB, Nelson RW, Scott-Moncrieff JC, Neal L, Bottoms GD. Serial thyroid hormone concentrations in healthy euthyroid dogs, dogs with hypothyroidism, and euthyroid dogs with atopic dermatitis. *Br Vet J.* 1992;148:451-458.
47. Reimers TJ, Lawler DF, Sutaria PM, Correa MT, Erb HN. Effects of age, sex, and body size on serum concentrations of thyroid and adrenocortical hormones in dogs. *Am J Vet Res.* 1990;51:454-457.
48. Gaughan KR, Bruyette DS. Thyroid function testing in greyhounds. *Am J Vet Res.* 2001;62:1130-1133.
49. Daminet S, Ferguson DC. Influence of drugs on thyroid function in dogs. *J Vet Intern Med.* 2003;17:463-472.
50. Kantrowitz LB, Peterson ME, Melián C, Nichols R. Serum total thyroxine, total triiodothyronine, free thyroxine, and thyrotropin concentrations in dogs with nonthyroidal disease. *J Am Vet Med Assoc.* 2001;219:765-769.

51. Coccaro EF, Kavoussi RJ, Hauger RL. Serotonin function and antiaggressive response to fluoxetine: a pilot study. *Biol Psychiatry*. 1997;42: 546-552.
52. Peremans K, Audenaert K, Coopman F, et al. Estimates of regional cerebral blood flow and 5-HT_{2A} receptor density in impulsive, aggressive dogs with ^{99m}Tc-ECD and ¹²³I-5-I-R91150. *Eur J Nucl Med Mol Imaging*. 2003;30:1538-1546.
53. Sanner JE, Frazier L, Udtha M. Effects of delayed laboratory processing on platelet serotonin levels. *Biol Res Nurs*. 2013;15:13-16.
54. Audhya T, Adams JB, Johansen L. Correlation of serotonin levels in CSF, platelets, plasma, and urine. *Biochim Biophys Acta*. 2012;1820: 1496-1501.
55. Reisner IR, Mann JJ, Stanley M, Huang YY, Houpt KA. Comparison of cerebrospinal fluid monoamine metabolite levels in dominant-aggressive and non-aggressive dogs. *Brain Res*. 1996;714:57-64.
56. León M, Rosado B, García-Belenguer S, Chacón G, Villegas A, Palacio J. Assessment of serotonin in serum, plasma, and platelets of aggressive dogs. *J Vet Behav Clin Appl Res*. 2012;7:348-352.
57. Matheson GJ, Schain M, Almeida R, et al. Diurnal and seasonal variation of the brain serotonin system in healthy male subjects. *Neuroimage*. 2015;112:225-231.
58. Bruschetta G, Di Pietro P, Miano M, et al. Daily variations of plasma serotonin levels in 2-year-old horses. *J Vet Behav Clin Appl Res*. 2013;8:95-99.
59. Pietraszek MH, Urano T, Sumiyoshi K, et al. Diurnal variations of whole blood serotonin content in patients with depression and neurosis. *J Neurol Neurosurg Psychiatry*. 1992;55:336.
60. Emiliano ABF, Fudge JL. From galactorrhea to osteopenia: rethinking serotonin-prolactin interactions. *Neuropsychopharmacology*. 2004;29: 833-846.
61. New AS, Trestman RF, Mitropoulou V, et al. Low prolactin response to fenfluramine in impulsive aggression. *J Psychiatr Res*. 2004;38: 223-230.
62. Mullur R, Liu Y-Y, Brent GA. Thyroid hormone regulation of metabolism. *Physiol Rev*. 2014;94:355-382.
63. Zetting G, Asenbaum S, Fueger BJ, et al. Increased prevalence of subclinical brain perfusion abnormalities in patients with autoimmune thyroiditis: evidence of Hashimoto's encephalitis? *Clin Endocrinol (Oxf)*. 2003;59:637-643.
64. Müssig K, Leyhe T, Holzmüller S, et al. Increased prevalence of antibodies to central nervous system tissue and gangliosides in Hashimoto's thyroiditis compared to other thyroid illnesses. *Psychoneuroendocrinology*. 2009;34:1252-1256.

How to cite this article: Hrovat A, De Keuster T, Kooistra HS, et al. Behavior in dogs with spontaneous hypothyroidism during treatment with levothyroxine. *J Vet Intern Med*. 2018;1-8.
<https://doi.org/10.1111/jvim.15342>